

We claim:

1           1.       An improvement in a method for assessing protein activity in a cell,  
2   portion of a cell, or group of cells comprising introducing four or more reporter  
3   molecules of protein activity in the cell, portion of a cell, or group of cells.

1           2.       The improvement of Claim 1 where introducing reporter molecules  
2   of protein activity in a cell, portion of a cell, or group of cells comprises  
3   introducing five or more reporter molecules of protein activity in the cell, portion  
4   of a cell, or group of cells.

1           3.       The improvement of Claim 1 where introducing reporter molecules  
2   of protein activity in a cell, portion of a cell, or group of cells comprises  
3   introducing six or more reporter molecules of protein activity in the cell, portion of  
4   a cell, or group of cells.

1           4.       The improvement of Claim 1 where introducing reporter molecules  
2   of protein activity in a cell, portion of a cell, or group of cells comprises  
3   introducing ten or more reporter molecules of protein activity in the cell, portion of  
4   a cell, or group of cells.

1           5.     A method for profiling signal transduction pathways comprising:  
2     making a single data measurement of proteins in a cell, portion of a cell or group  
3     of cells; and  
4     detecting or quantifying protein activity of three or more proteins in a cell, portion  
5     of a cell or group of cells.

1           6.     The method of claim 5 where detecting or quantifying protein  
2     activity comprises detecting or quantifying protein activity of four or more proteins  
3     in a cell, portion of a cell or group of cells.

1           7.     The method of claim 5 where detecting or quantifying protein  
2     activity comprises detecting or quantifying protein activity of five or more proteins  
3     in a cell, portion of a cell or group of cells.

1           8.     The method of claim 5 where detecting or quantifying protein  
2     activity comprises detecting or quantifying protein activity of six or more proteins  
3     in a cell, portion of a cell or group of cells.

1           9.     The method of claim 5 where detecting or quantifying protein  
2     activity comprises detecting or quantifying protein activity of ten or more proteins  
3     in a cell, portion of a cell or group of cells.

1           10.       A method of detecting protein activity in a cell, portion of a cell, or  
2 group of cells comprising:  
3           introducing into the cell, portion of a cell, or group of cells reporter  
4 molecules which identify one or a plurality of protein activities or respond to  
5 protein activity in the cell, portion of a cell, or group of cells;  
6           releasing the reporter molecules from the cell, portion of a cell, or group of  
7 cells; and  
8           sensing the reporter molecules to identify one or a plurality of protein  
9 activities.

1           11.       The method of claim 10 further comprising:  
2           recording the protein activity indicated by the reporter molecules; and  
3           compiling a tabulation of protein activity corresponding to the state of protein  
4 activity within the cell, portion of a cell, or group of cells.

1           12.       The method of claim 10 further comprising exposing the cell,  
2 portion of a cell, or group of cells to said external stimulus or stimuli prior to  
3 releasing the reporter molecules from the cell, portion of a cell, or group of cells.

1           13.       The method of Claim 12, wherein exposing the cell, portion of a  
2 cell, or group of cells to said external stimulus or stimuli comprises exposing the  
3 cell, portion of a cell, or group of cells to a pharmaceutical compound.

1           14.       The method of Claim 13, wherein compiling a tabulation of protein  
2   activity corresponding to the external stimulus or stimuli comprises compiling a  
3   tabulation of cellular protein activity responsive to the pharmaceutical compound.

1           15.       The method of Claim 13, wherein compiling a tabulation of protein  
2   activity corresponding to the external stimulus or stimuli comprises compiling a  
3   map of cellular response to the pharmaceutical compound.

1           16.       The method of Claim 13, wherein releasing the reporter molecules,  
2   sensing the released reporter molecules, recording the protein activity indicated  
3   by the reporter molecules, and compiling a tabulation of protein activity  
4   corresponding to the external stimulus or stimuli comprises releasing, sensing,  
5   recording, and compiling at least one of desired cellular response and an  
6   undesired other response.

1           17.       The method of Claim 10 wherein introducing into the cell, portion of  
2   a cell, or group of cells reporter molecules which identify one or a plurality of  
3   protein activities comprises introducing the reporter molecules into the cell,  
4   portion of a cell, or group of cells by attachment to an auxiliary molecule or  
5   polymer which is taken up into the cell, portion of a cell, or group of cells.

18. The method of Claim 17 wherein introducing the reporter molecules into the cell, portion of a cell, or group of cells by attachment to an auxiliary molecule or polymer comprises introducing the reporter molecules into the cell, portion of a cell, or group of cells by attachment of a peptide or peptide analog.

19. The method of Claim 10 further comprising labeling the reporter molecules to facilitate sensing thereof.

20. The method of Claim 19 wherein labeling the reporter molecules to facilitate sensing thereof comprises labeling the reporter molecules with at least one of the constituents selected from the group consisting of a fluorescent group, stable or radioactive isotope, or biotin.

21. The method of Claim 19 wherein labeling the reporter molecules with at least one of the constituents selected from the group consisting of a fluorescent group, stable or radioactive isotope, or biotin comprises labeling each of the reporter molecules with same label.

22. The method of Claim 19 wherein labeling the reporter molecules with at least one of the constituents selected from the group consisting of a fluorescent group, stable or radioactive isotope, or biotin comprises labeling each of the reporter molecules with different labels.

1           23.       An improvement in a method for detecting protein activity in a cell,  
2       portion of a cell, or group of cells which includes a native substrate at a  
3       physiologic concentration, the improvement comprising introducing into the cell,  
4       portion of a cell, or group of cells reporter molecules at a subphysiologic  
5       concentration compared with the concentration of a native substrate, which  
6       reporter molecules identify one or a plurality of protein activities.

1           24.       The method of Claim 10 where introducing into the cell, portion of a  
2       cell, or group of cells reporter molecules which identify one or a plurality of  
3       protein activities comprises introducing the reporter molecules in which at least  
4       one has a concentration of less than or equal to 10 micromolar.

1           25.       The method of Claim 10 where introducing into the cell, portion of a  
2       cell, or group of cells reporter molecules which identify one or a plurality of  
3       protein activities comprises introducing the reporter molecules in which at least  
4       one has a concentration of less than or equal to 1 micromolar.

1           26.       The method of Claim 10 where introducing into the cell, portion of a  
2       cell, or group of cells reporter molecules which identify one or a plurality of  
3       protein activities comprises introducing the reporter molecules in which at least  
4       one has a concentration of less than or equal to 100 nanomolar.

1           27.       The method of Claim 10 further comprising diminishing or  
2   terminating a chemical reaction involving the reporter molecule by liberating the  
3   reporter molecule and an altered reporter molecule from the cell, portion of a cell,  
4   or group of cells.

1           28.       The method of Claim 27 wherein diminishing or terminating a  
2   chemical reaction involving the reporter molecule comprises diminishing or  
3   terminating said chemical reaction by dilution.

1           29.       The method of Claim 10 further comprising diminishing or  
2   terminating a chemical reaction involving the reporter molecule by the use of  
3   scavengers or inhibitors.

1           30.       The method of Claim 10 further comprising labeling said reporter  
2   molecule and stopping a chemical reaction involving said reporter molecule by  
3   introducing an unlabeled reporter molecule.

1           31.       The method of Claim 10 further comprising stopping a chemical  
2   reaction involving the reporter molecule before releasing the reporter molecule  
3   and an unaltered reporter molecule from the cell, portion of a cell, or group of  
4   cells.

1           32.       The method of Claim 29 wherein diminishing or terminating a  
2 chemical reaction involving the reporter molecule by the use of scavengers or  
3 inhibitors comprises introducing the scavenger; or inhibitor photochemically from  
4 a caged scavenger or caged inhibitor.

1           33.       The method of Claim 10 further comprising stopping a chemical  
2 reaction involving the reporter molecule after releasing the reporter molecule  
3 and/or an unaltered reporter molecule from the cell, portion of a cell, or group of  
4 cells.

1           34.       The method of Claim 33 wherein the time interval between  
2 releasing the reporter molecule and/or altered reporter molecule from the cell,  
3 portion of a cell, or group of cells and stopping a chemical reaction involving the  
4 reporter molecule is less than 1 second.

1           35.       The method of Claim 33 wherein the time interval between  
2 releasing the reporter molecule and/or altered reporter molecule from the cell,  
3 portion of a cell, or group of cells and stopping a chemical reaction involving the  
4 reporter molecule is less than 33 milliseconds.

1           36.       The method of Claim 33 wherein the time interval between  
2 releasing the reporter molecule and/or altered reporter molecule from the cell,



3 portion of a cell, or group of cells and stopping a chemical reaction involving the  
4 reporter molecule is less than 10 microseconds.

1 37. The method of Claim 10 further comprising distinguishing between  
2 the reporter molecule and an altered reporter molecule by electrophoresis,  
3 microchromatography, mass spectroscopy, fluorescence polarization  
4 spectroscopy, or an affinity array.

1 38. The method of Claim 10 further comprising distinguishing between  
2 the reporter molecule and/or an altered reporter by fluorescence spectroscopy,  
3 polarization techniques, mass spectroscopy, conductivity, or radioactive  
4 detection.

1 39. The method of Claim 10 further comprising detecting reporter  
2 molecules and/or altered reporter molecules using two dimensional gel  
3 electrophoresis, protein mass spectroscopy, yeast 2-hybrid assays, structural  
4 biology, intracellular ion and other indicators, intracellular protein location  
5 techniques, DNA arrays, or flow cytometry, including sheathed and unsheathed  
6 flow and flow cytometry on a microfluidics device or combinations thereof.

1 40. The method of Claim 10 further comprising performing in  
2 combination detecting reporter molecules and/or altered reporter molecules using

3 two dimensional gel electrophoresis, protein mass spectroscopy, yeast 2-hybrid  
4 assays, structural biology, intracellular ion and other indicators, intracellular  
5 protein location techniques, DNA arrays, or flow cytometry, including sheathed  
6 and unsheathed flow and flow cytometry on a microfluidics device.

1 <sup>37</sup>  
40. The method of Claim 10 wherein sensing the reporter molecules  
2 comprises detecting and/or quantifying protein activity of three or more proteins.

1 <sup>38</sup>  
41. The method of Claim 10 wherein sensing the reporter molecules  
2 comprises detecting and/or quantifying protein activity of four or more proteins.

1 <sup>39</sup>  
42. The method of Claim 10 wherein sensing the reporter molecules  
2 comprises detecting and/or quantifying protein activity of five or more proteins.

1 <sup>40</sup>  
43. The method of Claim 10 wherein sensing the reporter molecules  
2 comprises detecting and/or quantifying protein activity of six or more proteins.

1 <sup>41</sup>  
44. The method of Claim 10 wherein sensing the reporter molecules  
2 comprises detecting and/or quantifying protein activity of ten or more proteins.

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45.

1 An apparatus for measuring the protein activity in a cell, portion of a  
2 cell, or group of cells that have been altered by introduction of a reporter  
3 molecule of one or a plurality of proteins comprising:  
4 means for lysing the cell, portion of a cell, or group of cells;  
5 means for collecting the contents of a cell or group of cells or a portion of  
6 the contents of a cell or group of cells; and  
7 means for distinguishing reporter molecules and altered reporter molecules;  
8 means for sensing reporter molecules and/or altered reporter molecules.

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1 The apparatus of claim 45 further comprising:  
2 means of recording the protein activity indicated by the reporter molecules;  
3 and  
4 means of compiling a tabulation of protein activity.

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1 The apparatus of Claim 45 further comprising means for introducing  
2 reporter molecules into the cell, portion of a cell, or group of cells.

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48.

1 The apparatus of Claim 47 where the means for introducing  
2 reporter molecules comprises means for microinjection, optoinjection,  
3 optoporation, electroporation, or attachment of an auxiliary molecule causing the  
4 reporter to pass into the cell, portion of a cell, or group of cells.

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1     ~~49.~~     The apparatus of Claim 45 where the means for introducing  
2     reporter molecules comprises means for microinjection, optoinjection,  
3     optoporation, electroporation, or attachment of an auxiliary molecule causing the  
4     reporter to pass into the cell, portion of a cell, or group of cells.

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1     ~~50.~~     The apparatus of Claim 45 further comprising means for presenting  
2     a cell, portion of a cell, or group of cells to a collection device.

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1     ~~51.~~     The apparatus of Claim 50 where the means for presenting  
2     comprises one means selected from the group consisting of a multi-well plate, a  
3     dielectrophoresis trap, laser tweezers, a microlumen or an array of microlumens.

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1     ~~52.~~     The apparatus of Claim 45 where the means for collecting  
2     comprises means for aspiration through a microlumen.

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1     ~~53.~~     The apparatus of Claim 45 where the means for collecting  
2     comprises one means selected from the group of a microlumen, microwell,  
3     nanowell, picowell, or microfluidics chip.

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1     ~~54.~~     The apparatus of Claim 45 where the means for lysing comprises  
2     one means selected from the group consisting of a laser, means for providing a  
3     shock wave, means for providing an electric field, means for treating the cell or

4 group of cells or a portion of the contents of a cell or group of cells with a  
5 chemical reagent.

1 <sup>56</sup>  
55. The apparatus of Claim 45 where the means for distinguishing  
2 comprises one means selected from the group consisting of means for using  
3 electrophoresis, means for using microchromatography, means for using mass  
4 spectroscopy, means for using affinity arrays or a combination thereof.

1 <sup>57</sup>  
56. The apparatus of Claim 55 where the means for using affinity  
2 arrays comprises an array of biomolecules selected from the group consisting of  
3 DNA, RNA, PNA, proteins, receptors, enzymes, antibodies or combinations  
4 thereof.

1 <sup>58</sup>  
57. The apparatus of Claim 45 where the means for distinguishing  
2 comprises means for performing electrophoresis conducted on a microfluidics  
3 device.

1 <sup>59</sup>  
58. The apparatus of Claim 45 where the means for sensing comprises  
2 one means selected from the group consisting of means for using fluorescence  
3 spectroscopy, means for using mass spectroscopy, means for using conductivity,  
4 or means for using radioactive detection.

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1 The apparatus of Claim 45 further comprising a computer controlled  
2 collection device.

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1 The apparatus of Claim 45 further comprising a computer-  
2 controlled lysis device.

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1 The apparatus of Claim 45 further comprising a computer-  
2 controlled distinguishing device.

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1 The apparatus of Claim 45 further comprises a computer-controlled  
2 sensing device.

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1 The apparatus of Claim 45 further comprising a data processor  
2 coupled to the means for sensing to record changes in the reporter molecules.

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1 The apparatus of Claim 45 further comprising a data processor to  
2 compile a tabulation of protein activities.